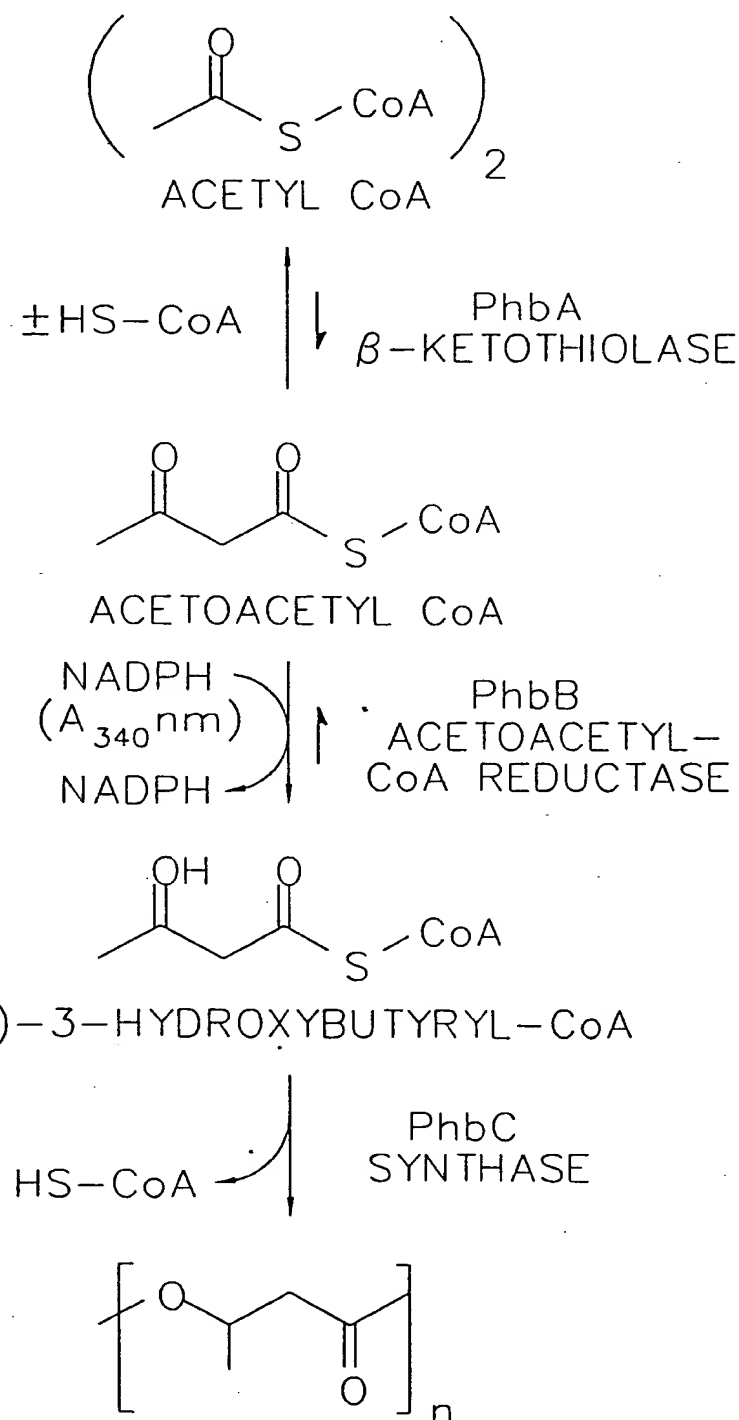


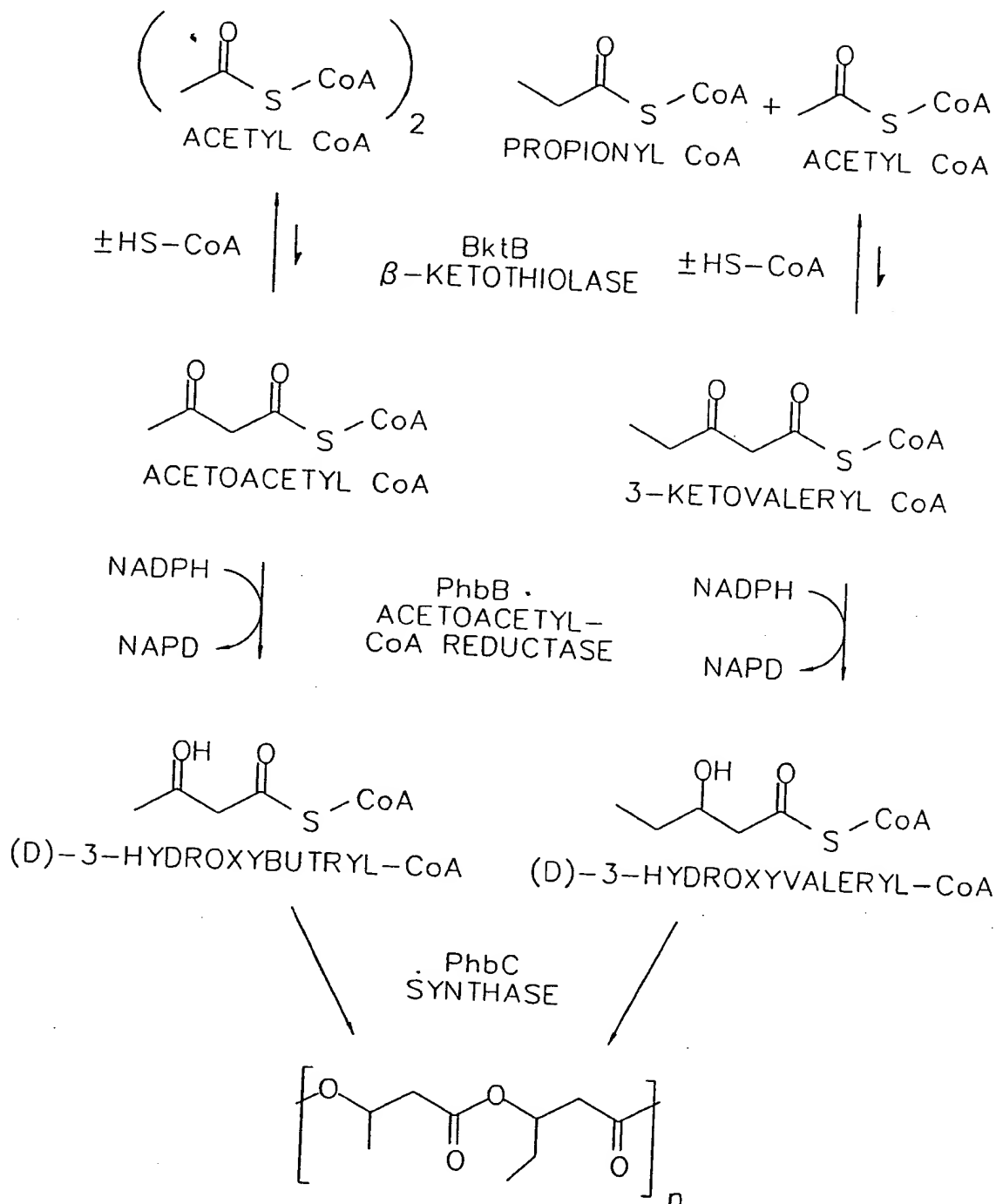
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FIG. 1



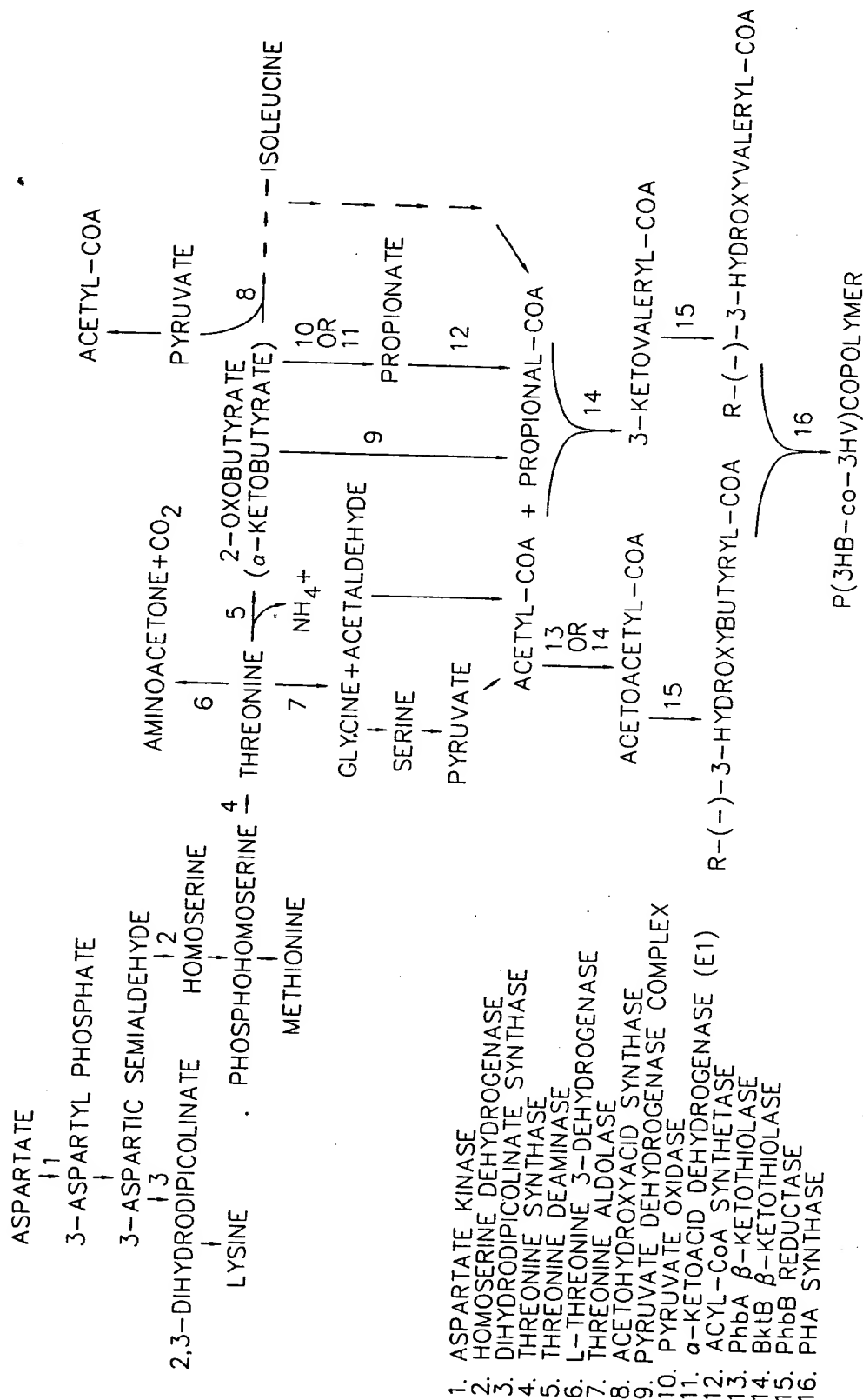
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FIG. 2



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FIG. 3



1. ASPARTATE KINASE
2. HOMOSERINE DEHYDROGENASE
3. DIHYDRODIPICOLINATE SYNTHASE
4. THREONINE SYNTHASE
5. THREONINE DEAMINASE
6. L-THREONINE ALDOLASE
7. THREONINE 3-DEHYDROGENASE
8. ACETOHYDROXYACID SYNTHASE
9. PYRUVATE DEHYDROGENASE COMPLEX
10. PYRUVATE OXIDASE
11. α-KETOACID DEHYDROGENASE (E1)
12. ACYL-CoA SYNTHETASE
13. PhbA β-KETOTHIOLASE
14. BktB β-KETOTHIOLASE
15. PhbB REDUCTASE
16. PHA SYNTHASE

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FIG. 4

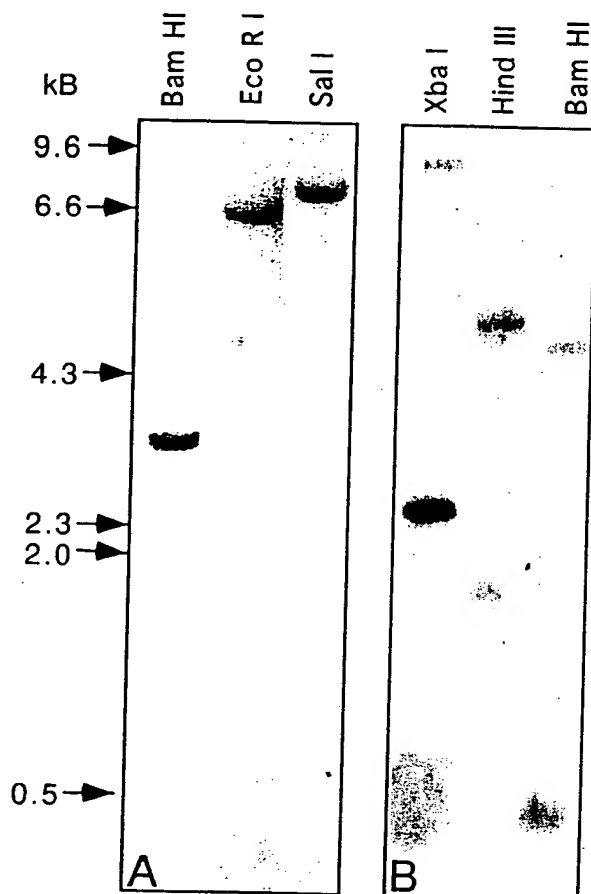
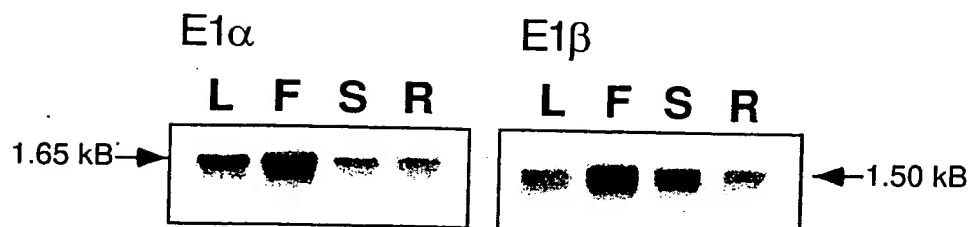


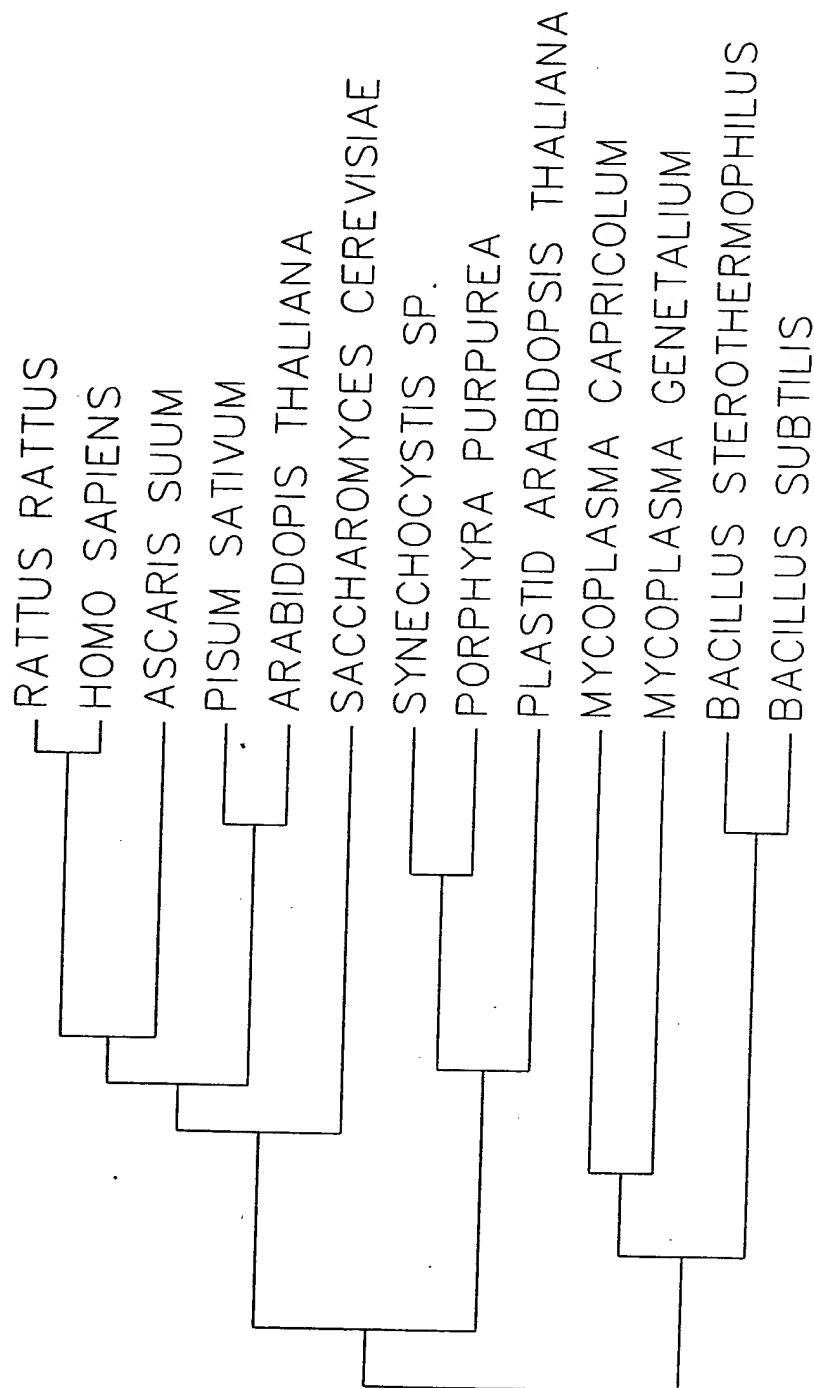
FIG. 5



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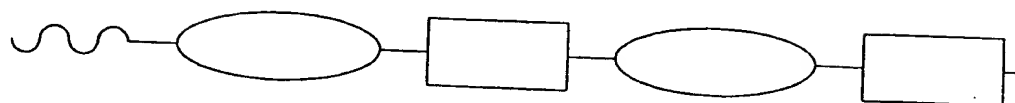
FIG. 6A



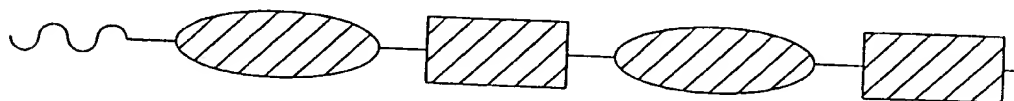
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FIG. 7B

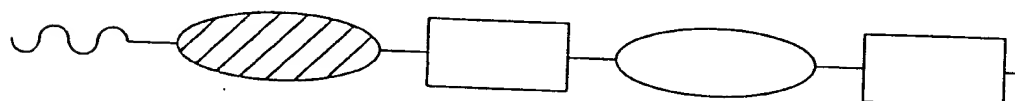
BRANCHED-CHAIN E1 β



PLASTID E1 β



PLASTID TARGETED BRANCHED-CHAIN E1 β CHIMERA



CONSTRUCT 2: REPLACE THE N-TERMINUS OF THE BRANCHED-CHAIN E1 β (INCLUDING THE E2 BINDING DOMAIN) WITH THE N-TERMINUS OF THE PLASTID E1 β (INCLUDING THE CHLOROPLAST TARGETING PEPTIDE AND THE PLASTID E2 BINDING DOMAIN). THIS CREATES A PLASTID TARGETED BRANCHED-CHAIN E1 β CHIMERA.

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FIG. 7C

BRANCHED-CHAIN E2



PLASTID E2



PLASTID TARGETED BRANCHED-CHAIN E2

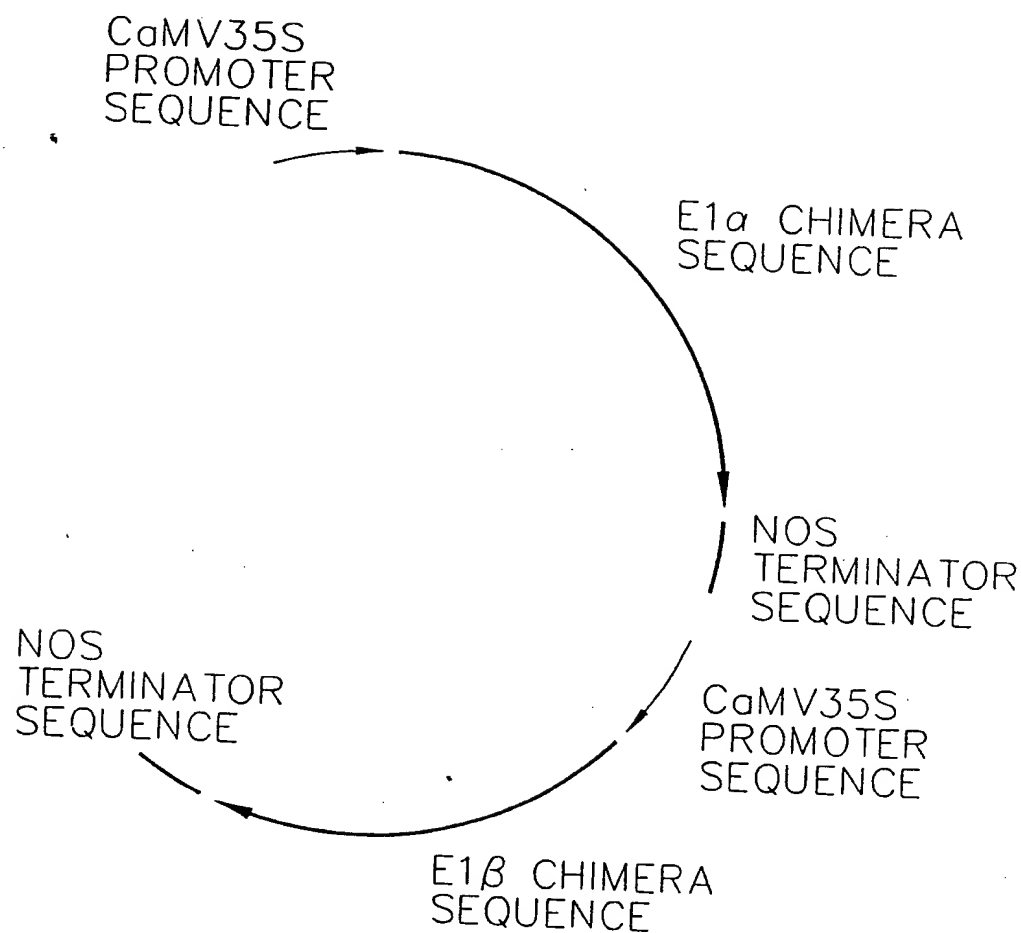


CONSTRUCT 3: ATTACH THE CHLOROPLAST TARGETING PEPTIDE OF THE PLASTID E2 TO THE MATURE PORTION OF THE BRANCHED-CHAIN E2, TO CREATE A PLASTID TARGETED BRANCHED-CHAIN E2 CHIMERA.

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FIG. 7D

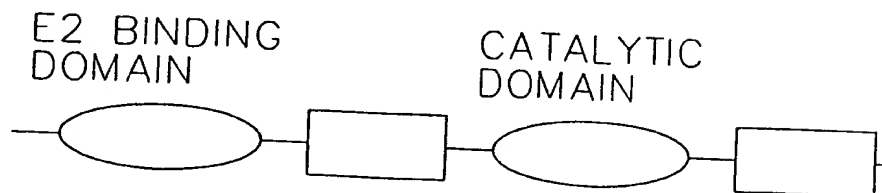


CONSTRUCT 4: MEGA PLASMID CODING FOR BOTH CHIMERIC (PLASTID TARGETED BRANCHED-CHAIN) SUBUNITS OF THE PDH. ATTACH THE E1 α CHIMERIC SEQUENCE TO THE E1 β CHIMERIC SEQUENCE WITH TRANSCRIPTION TERMINATOR AND PROMOTER SEQUENCES BETWEEN THE TWO.

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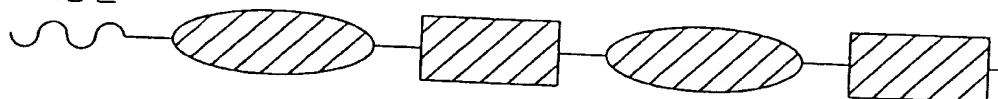
FIG. 7E

BRANCHED-CHAIN E1 β

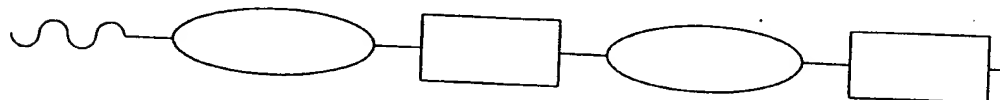


CHLOROPLAST
TARGETING
PEPTIDE

PLASTID E1 β



PLASTID TARGETED BRANCHED-
CHAIN E1 β CHIMERA



CONSTRUCT 5: ATTACH THE CHLOROPLAST TARGETING PEPTIDE OF THE PLASTID E1 β TO THE MATURE PORTION OF THE BRANCHED-CHAIN E1 β . THIS CREATES A PLASTID TARGETED BRANCHED-CHAIN E1 β CHIMERA.

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FIG. 8A

Plastid A.t.	MATAFAPTKLTATVPLHGSHENRLLLPIRLAPPSSFLGSTRSLSLRRLNH	50
<i>P.purpurea</i>	-----	
<i>A.thaliana</i>	-----MALSRSSRSNIITRPFSAAFSRLIS	26
<i>H.sapiens</i> II	-----MRKMLAAVSRVLSGASQKPASRVLVAS	27
<i>S.cerevisiae</i>	---MLAASFQRQPSQLVRGLGAVLRTPTRIGHVRTMATLKTDDKKAPEDI	47
<i>A.suum</i> I	-----MIFVFANIFKVPTVSPSVMAISV	23
<i>M.capricolum</i>	-----MTYL	4
<i>B.subtilis</i>	-----MGVKTFQFPFAEQL	14
Consensus	-----	50

Motif 1

SNATRRSPVVSQEVVKEQSTNNTSLITKEEGLELYEDMILGRSFEDM	100
-----MSYPKKVELPLTNCNQINLTCHKLLVLYEDMLLGRNFEDM	40
TDTPITITETSLPFTAHLCDPPSRVSESSQELLD-FFRTMALMRRMEIA	75
RNFANDATFEIKKCDLHRLEEGPPVTTVLTREDGLKYRMMQTVRRMELK	77
EGSDTVQIELPESSFESYMLEPPDLSYETSKATLLQMYKDMVIRRMEMA	97
RLASTEATFQTKPFKLHKLDSPDINVHVTKEAVHYTQMLTIRRMESA	73
GKFDPLKNEKVCVLDKDGKVINPKLMPKISDQEI LEAYKIMNLSRRQDIY	54
EKVAEQFPFTFQILNEEGEVVNEEAMPFELSDEQLKE-LMRRMVYTRILDQR	63
.....L..Y..M...RR.E..	100

o

CAQMYRGRKMFGFVHLYNGQEAVSTGF I KLLTKSDSVVSTYRDHVALSK	150
CAQMYRGRKMFGFVHLYNGQEAVSTGV I KLLSDKYVCSTYRDHVALSK	90
ADSLYKANVIRGFCHLYDGQEAVAIGMEAAITKKDAIITAYRDHCFILGR	125
ADQLYKQKIIRGFCHLCDGQEACCVGLEAGINPTDHLITAYRAHGFTFTR	127
CDALYKAKKIRGFCHLSVGQEAI AVGIENAITKLDSIITSYRCHGFTFMR	147
AGNLYKEKKVRGFCHLYSGQEACAVGTKAAMDAGDAAVTAYRCHGWYLS	123
QNTMQRQGRLLSFLSSTGQEACEVAYINALNKKTDHFVSGYRNNAAWLAM	104
SISLNRQGRL-GFYAPTAGQEASQIASHFALEKEDFILPGYRDVPQIIWH	112
...LY.....GF.HL..GQEA...G.....K.D.....YR.H.....	150

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FIG. 8B

TPP-binding site

GVSARAVMSELFVKVTGCCRGQGGSMHMF SKEHNMLGGFAF IGEGIPVAT	200
GVPSQNVMAELFGKETGCSRGRGGS MHIF SAPHNFLGGFAFIAEGIPVAT	140
GGSLHEVFS ELMGRQAGCSKGKGGSMH FYKKESSFYGGHGIVGAQVPLGC	175
GLSVREILAE LTGRKGGCAKGKGGSMHMYAKN - - FYGGNGIVGAQVPLGA	175
GASVRAVLAELMGRRAGVSYGKGGSMHLYAPG - - FYGGNGIVGAQVPLGA	195
GSSVAKVLC ELTGRITGNVYGKGGSMHMYGEN - - FYGGNGIVGAQQPLGT	171
GQLVRNIMLYWIGNEAG - GK APEG - VNCLPPN - - - - - IVIGSQYSQAT	145
GLPLYQAFLFSRGHFHG - NQIPEG - VNVLP PQ - - - - - IIIGAQYIQAA	153

G.S...V...EL.G...G...G.GGSMH.....-F.GG..I.GAQ.P...	200
--	-----

PDH β binding site

GAAFSSKYRREVLKQDCD - DVTVAFFGDGTCNNGQFFEC LNMAALYKLPI	249
GAAFQSIYRQQLKEP GELRV TACFFGDGTTNNGQFFEC LNMAVLWKLPI	190
GIAFAQKYNKE - - - EA - - - - VTFALYGDGAANQGQLFEALN I SALWDLPA	218
GIALACKYNGK - - - DE - - - - VCLTLYGDGAANQGQ IFEAYNMAALWKLPC	218
GLAFAHQYKNE - - - DA - - - - CSFTLYGDGASNQGQVFESFNMAKLWNL PV	238
GIAFAMKYRKE - - - KN - - - - VCITMFGDGATNQGQLFESMNMAKLWDL PV	214
GIAFADKYRKT - - - GG - - - - VVVT TTDGGSSEGETYEAMNFAKLHEVPC	188
GVALGLKMRGK - - - KA - - - - VAITYTGDGGS SQDFYEGINFAGAFKAPA	196

G.AFA.KYR.....-V..T..GDG..NQGQ.FE..NMA.LW.LP.	250
---	-----

*3

IFVVENNLWAIGMSHLRATSDPEIWKKGPAFGMPGVHVDGMDVLKVREVA	299
IFVVENNQWAIGMAHHRSSSIPEIHKKAEAFGLPGIEVDGMDVLAVRQVA	240
ILVCENNHYGMGTAEWRAAKSPSYKRGD - Y - VPGLKVDGMDAFVAVKQAC	266
IFICENNR YGMGT SVERAASTDYKRGD - F - IPGLRVDGMDILCVREAT	266
VFCCENNKYGMGTAAARSSAMTEYFKRGQ - Y - IPGLKVNGMDILAVYQAS	286
LYVCENNGYGMGTAAARSSASTDY YTRGD - Y - VPGI WVDGMDVLAVRQAV	262
IFVIENNKWAISTARSEQTKSINFAVKGIATGIPSIIVDGN DY LACIGVF	238
IFVVQNNRFAISTPVEKQTVAKTLAQKAVAAGIPGIQVDGMDPLAVYAAV	246

IFV.ENN....GTA..R.....K.G.....PG..VDGMD.LAV...A.	300
--	-----

*1 . 2

KEAVTRARRGEGPTLVECETYRFRGHSLADPD - ELRDAAE - KAKYAARDP	347
EKAVERARQGQPTLIEALTYRFRGHSLADPD - ELRSRQE - KEAWVARDP	288
KFAKQHALE - KGPIILEMDTYRYHGHSMSDPGSTYRTRDEISGVRQERDP	315
RFAAAYCRSGKGPILMELQTYRYHGHSMSDPGVSYRTREEIQEVRSKSDP	316
KFAKDWCLSGKGPLVLEYETYRYGGHSMSDPGTTYRTRDEIQHMRSKNDP	336
RWAKEWCNAGKGPLMIEMATYRYSGHSMSDPGSTSYRTREEVQEVKTRDP	312
KEVVEYVRKGNPVLVECDTYRLGAHSSSDNPDAYRPKGEFEEM - AKFDP	287
KAARERAINGEGPTLIETLCFRYGPHTMSGDDPTRYRSKELENEWAKKDP	296

K.A.....G.GP.L.E..TYRY.GHSMSDP...YR.R.E.....DP	350
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FIG. 8C

IAALKKYLIENKLAKEAELKSIEKKIDELVEEAVEFADASPQPG--RSQL	395
IKKLKKHILDNQIASSDELNDIQSSVKIDLEQSVFAMSSPEPN--ISEL	336
IERIKKLVLSHDLATEKELKDMEKEIRKEVDDAIKAKDCPMPE--PSEL	363
IMLLKDRMVNSNLASVEELKEIDVEVRKEIEDAAQFATADPEPP--LEEL	364
IAGLKMHLIDLGIATEAEVKAYDKSARKYVDEQVELADAAPPPEAKLSIL	386
ITGFKDKIVTAGLVTEDEIKEIDKQVRKEIDAAVKQAHTDKESPVELMLT	362
LIRLKQYLIDKKIWSDEQQAQLEAEQDKFVADEFWVEKNKNYDL-IDIF	336
LVRFRKFLEAKGLWSEEEENNVIQAKEEIKEAIIKKADETPKQK--VTDL	344
I..LK.....LA.E.E.K.....K....A...A...P.P.----L	400
LENVFADPKGFGIGPDGRYRCEDPKFTEG-TAQV	428
-----K-----RY-----LFADN-----	344
FTNVYV--KGFG---TESFGPDRKEVKAS-LP--	389
GYHIYSSDPPF----EVRGANQWIKFKSVS----	390
FEDVYVKGTTETPTLRGRIPEDTWDFKKQGFASRD	420
DIYYNTPAQYVRCTTDEVLQKYLTSEEAVKALAK	396
KYQYDKMDIFLEEYKEAKEFFEKYPESKEGGHH	370
ISIMFE-ELPF-----NLKEQYEIYKEKESK--	369
.....	434

336 363 364 386 362 336 344 400 428 344 389 390 420 396 370 369 434

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GAAMTGLRPVIEGMNMGFLLAFNQISNNCGMLHYTSGGQFTIPVVIRGP	200
GAAITGLRPIVEGMNMSFLLAFNQISNNAGMLRYTSGGNFTLPLVIRGP	118
GAAYAGLKPVVEFMTFNFSMQAIDHIINSAAKSNYMSAGQINVPIVFRGP	150
GAAMAGLRPICEFMTFNFSMQAIDQVINSAAKTYMSGGLQPVPVIFRGP	147
GAALKGLKPIVEFMSFNFSMQAIDHVVNSAAKTHYMSGGTQKCQMVFRGP	153

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FIG. 9B

GAAMNGLRPICEFMSMNFSMQGIDHIINSAAKAHYMSAGR FHVPIVFRGA 148
GMAMNGMKPVLEMQFEGLGLASLQNI FTNISRMNRTRGKYTAPMVI RMP 118
GLALQGFRPVPEIQFFGFVYEVMD SICQMARIYRTGGRYHMPITIRSP 118

GAA..GLRP..E.M...F...A.D.I.N.AA...Y.SGG....P.V.RGP 200

Region 2

Region 2	
GGVGRQLGAEHSQRLESYFQSIPIGIQMVACSTPYNAKGLMKAAIRSENPV	250
GGVGRQLGAEHSQRLEAYFQAIPLGLKIVACSTPYNAKGLLKSAIRDNNPV	168
NGAAAGVGAQHSQCFAAWYASVPGLKVLAPYSAEDARGLLKAAIRDPDPV	200
NGASAGVAAQHSQCFAAWYGHCPLKVVSPWNSEDAKGLIKSAIRDNNPV	197
NGAAVGLGAQHSQDFSPWYGSIPGLKVLVPYSAEDARGLLKAAIRDPNPV	203
NGAAVGVAQQHSQDFTAWFMHCPGVKVVVPYDCEDARGLLKAAVRDDNPV	198
MGGGIRALEHHSEALEAVYAHIPGVQIVCPSTPYDTKGLILAAIDSPDPV	168
FGGGVHTPELHSDSLEGLVAQQPGLKVVIPSTPYDAKGLLISAIRDNDPV	168

.G.....A.HSQ...A.....PGLKVV.P.....DAKGLLKAAIRD.NPV 250

ILFEHVLLYN---	LKEKIPDEDI	CNLEE	AE	MVRPGEHITILTYSRMR	296
VFFEHVLLYN---	LQEEIPEDEYLI	PLDKA	E	VVRKGKDITILTYSRMRH	214
VFLENELLYGESFPI	SEEALDSSFC	LPIGKAKIEREGKDV	TI	VTFSKMGV	250
VVLENELMYGVPFE	FLPEAQSKDF	LPIGKAKIERQGT	HT	TVVSHSRPVG	247
VFLENELLYGESFEI	SEEALSPFTLPY-	KAKIEREGTDISIV	TYTRNVQ		252
ICLENEI	LYGMKFPVSPEAQSPDF	VL	PFGQAKIQRPGKDITIV	SLSIGVD	248
IVVEPTKLYR---	AFKQEV	PDEHYIVPIGEGYKIQEGNDL	TVV	TYGAQTV	215
IFLEHLKLYR---	SFRQEVPEGEYTI	PIGKADIKREGKDITI	IAYGAMVH		215

..LE..LLY.....E.....P.GKA.I.R.G.DITIVTYS..V. 300

Region 3

Region 3	
HVMQAAKTLVNK--GYDPEVIDIRSLKPFDLHTIGNSVKKTHRVLIVEEC	344
HVTEALPLLLND--GYDPEVLDLISLKPLDIDSISVSVKKKTHRVLIVEEC	262
FALKAAEKLAE--GISAEVINLRSIRPLDRATINASVRKTSRLVTVEEG	298
HCLEAAAVLSKE--GVECEVINMRTIRPDMETIEASVMKTNHLVTVEGG	295
FSLEAAAEILQKKY-GVSAEVINLRSIRPLDTEAIKTKVKKTNHLITVEST	301
VSLHAADELAKS--GIDCEVINLRCVRPLDFQTVKDSVIKTKHLVTVESG	296
DCQKAIALLKETHPNATIDLIDLRSIKPWDKKMVIESVKKTGRLLVVHEA	265
ESLKAAAELEKE--GISAEVVDLRTVQPLDIETIGSVEKTGRAIVVQEA	263

..L.AA..L...--G...EVI.LRS..PLD..TI..SV.KT.RL..VEE. 350

Region 4

MR TGGIGASLTAAINE-NFHDYLDAPVMCLSSQDVPTPYAGTLEEWTVVQ 393

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EAF FCHVPGIKVVI PRSPREAKG LLLSCIRDPNPVVF FE PKWLYRQAVEE	211
EAF FAHCPGIK VVI PRSPFQAKG LLLSCIEDKNPCIFFE PKILYRAAAEE	250
EAF FAHCPGIK VVV PRSPFQAKG LLLSCIEDKNPCIFFE PKILYRAAVEQ	249
EAF FAHCPGIK VVI PRSPFQAKG LLLSCIEDKNPCIFFE PKILYRAAVEE	250

